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Pharmacokinetic and pharmacodynamic bioequivalence of biosimilar MYL-1601D with US and European insulin aspart in healthy volunteers: A randomized, double-blind, crossover, euglycaemic glucose clamp study

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Abstract

Aim: To evaluate the pharmacokinetic (PK) and pharmacodynamic (PD) bioequivalence (BE) of MYL-1601D biosimilar with originator, NovoLog (Ref-InsAsp-US), and NovoRapid (Ref-InsAsp-EU).

Materials and Methods: This was a double-blind, randomized, crossover study that enrolled 71 healthy subjects to receive a single subcutaneous dose (0.2 U/kg) of each formulation under automated euglycaemic clamp conditions (ClampArt, level 81 mg/dL, duration 12 hours postdose). Primary PK endpoints were area under the plasma insulin aspart concentration-time curve from 0 to 12 hours ($AUC_{0\text{-}12h}$) and maximum plasma insulin aspart concentration (C_{max}). Primary PD endpoints were area under the glucose infusion rate (GIR) time curve from 0 to 12 hours ($AUC_{GIR0\text{-}12h}$) and maximum GIR (GIR_{max}). Insulin aspart in plasma was quantified using immunoaffinity purification followed by ultraperformance liquid chromatography and tandem mass spectrometric detection. The pairwise comparisons of geometric least square mean (LS-mean) ratio for a 90% confidence interval (CI) of primary PK, and 90% CIs (MYL-1601D vs. Ref-InsAsp-US) and 95% CIs (MYL-1601D vs. Ref-InsAsp-EU) of primary PD variables, were to be within 80% to 125% to show BE.

Results: MYL-1601D showed PK BE to both Ref-InsAsp-US ($AUC_{0\text{-}12h}$ geometric LS-mean ratio 102.17, 90% CI [100.26; 104.11]; C_{max} 106.13 [100.71; 111.85]) and Ref-InsAsp-EU ($AUC_{0\text{-}12h}$ 101.84 [100.04; 103.67]; C_{max} 105.74 [101.09; 110.60]). Likewise, MYL-1601D showed PD BE to Ref-InsAsp-US ($AUC_{GIR\_0\text{-}last}$ 99.93; 90% CI [95.74; 104.30]; $GIR_{\_max}$ 100.12 [94.46; 106.12]) and Ref-InsAsp-EU ($AUC_{GIR\_0\text{-}last}$ 96.42; 95% CI [91.17; 101.98]; $GIR_{\_max}$ 95.10 [89.37; 101.19]). All three insulin aspart products were well tolerated.

* A part of this study's data were presented as a poster presentation at the virtual 81st Scientific Sessions of the American Diabetes Association, held 25-29 June 2021.

Conclusion: MYL-1601D showed BE to Ref-InsAsp-US and Ref-InsAsp-EU with a comparable safety profile.

KEYWORDS
biosimilar, euglycaemic clamp study, insulin aspart, pharmacokinetics/pharmacodynamics

1 | INTRODUCTION

Insulin therapy is essential for the treatment of type 1 diabetes (T1D) and may be needed in type 2 diabetes (T2D). Currently available mealtime insulin analogues are effective, have a more physiological profile, and are associated with a lower risk of hypoglycaemia, but they are expensive.[1] Insulin aspart is a rapid-acting insulin marketed as NovoLog (Ref-InsAsp-US) in the United States and NovoRapid (Ref-InsAsp-EU) in the European Union (EU). It has a faster onset and a shorter duration of action, resulting in postprandial glycaemic control by means of lowering the total glucose excursion following a meal, in individuals with T1D and T2D.[2-5]

Insulin costs continue to rise and remain a concern for individuals with diabetes, their families, healthcare providers, insurers, and employers.[6] The American Diabetes Association advocates access to affordable and evidence-based insulin therapies in managing diabetes. The launch of rapid-acting insulin biosimilars should offer the advantages of mealtime insulin at reduced cost, thereby offering easy access to treatment for people with diabetes.[6]

MYL-1601D is a rapid-acting human insulin analogue being developed by Mylan (a Viatris company) as a biosimilar to Ref-InsAsp-US and Ref-InsAsp-EU (insulin aspart 100 U/mL). It is produced by recombinant DNA technology utilizing *Pichia pastoris* (yeast). MYL-1601D has been shown to be similar to Ref-InsAsp-EU in terms of the qualitative and quantitative composition of the active substance. In addition, the totality of the data evidence from in vitro pharmacology studies and comparative toxicity studies showed MYL-1601D to be similar to Ref-InsAsp-EU.[7] The objective of this clinical development programme was to show the pharmacokinetic (PK) and pharmacodynamic (PD) similarity of MYL-1601D to Ref-InsAsp-US and Ref-InsAsp-EU. The availability of MYL-1601D biosimilar may provide a more affordable treatment option for an individual with diabetes. This "pivotal" euglycaemic study was assessed by the European Medicines Agency (EMA) in December 2020 and was approved for marketing authorization by the EU Commission in 2021.[7]

This article presents the study findings of PK and PD variables of MYL-1601D insulin aspart in comparison with Ref-InsAsp-EU and Ref-InsAsp-US. Data from this study support the PK and PD evidence for the biosimilarity of MYL-1601D with Ref-InsAsp-EU and Ref-InsAsp-US in healthy subjects.

2 | MATERIALS AND METHODS

2.1 | Study design and treatment

This was a phase 1, randomized, double-blind, three-treatment, three-period, crossover, 12-hour euglycaemic glucose clamp study (registered at EudraCT; number: 2017-000770-12) conducted at two sites (Profil Neuss and Profil Mainz, Germany) in healthy subjects. The study had a screening visit, followed by three treatment periods, washout periods (at each treatment period), and a follow-up period after the last treatment (Figure 1). The study was conducted in accordance with the German Drug Law (Arzneimittelgesetz),[8] German Good Clinical Practice (GCP) Ordinance (GCP-Verordnung),[9] the Declaration of Helsinki,[10] and GCP guidelines.[11] The study protocol was approved by the local ethics committee and respective competent federal higher authority (German Federal Institute for Drugs and Medical Devices, BfArM). All subjects provided a signed informed consent form prior to any study-related activity.

Subjects were randomized to one of the six treatment sequences in a 1:1:1:1:1:1 ratio, receiving a single subcutaneous dose of 0.2 U/kg administration of one of the three study drugs on each dosing day (Figure 1). The planned study duration per subject was 20 to 69 days. Prior to dosing, the blood glucose (BG) clamp target level of 81 mg/dL was achieved using a variable intravenous (i.v.) infusion of human insulin.

2.2 | Subjects

To be eligible for the study, male and females with fasting plasma glucose levels of 5.5 mmol/L or less (≤100 mg/dL), aged 18-65 years, with a body mass index of 18.5-29.0 kg/m^2 at screening, were included. Key exclusion criteria included a history of allergies to insulin aspart, the presence of clinically significant concomitant medical conditions, clinically significant abnormal laboratory values, abnormal ECG findings, a history of alcoholism or drug abuse, or smoking more than five cigarettes per day.

2.3 | Study endpoints

The primary PK endpoints were area under the insulin aspart concentration-time curve from 0 to 12 hours ($AUC_{0\text{-}12h}$) and the maximum observed insulin aspart concentration (C_{max}). The secondary PK endpoints were area under the insulin aspart concentration-time curve from 0 to 4 hours ($AUC_{0\text{-}4h}$), from 0 to 6 hours ($AUC_{0\text{-}6h}$), from 6 to 12 hours ($AUC_{6\text{-}12h}$), and from 0 to infinity ($AUC_{0\text{-}\infty}$); time to maximum observed insulin aspart concentration (t_{max}); time to half-maximum before C_{max} ($t_{50\%\text{-}early}$); time to half-maximum after C_{max} ($t_{50\%\text{-}late}$); the terminal elimination half-life ($t_{1/2}$); and terminal elimination rate constant of insulin (λ_z). The primary PD endpoints were area under the glucose infusion rate from 0 hours until the end of clamp ($AUC_{GIR,0\text{-}last}$) and the maximum glucose infusion rate (GIR_{max}).

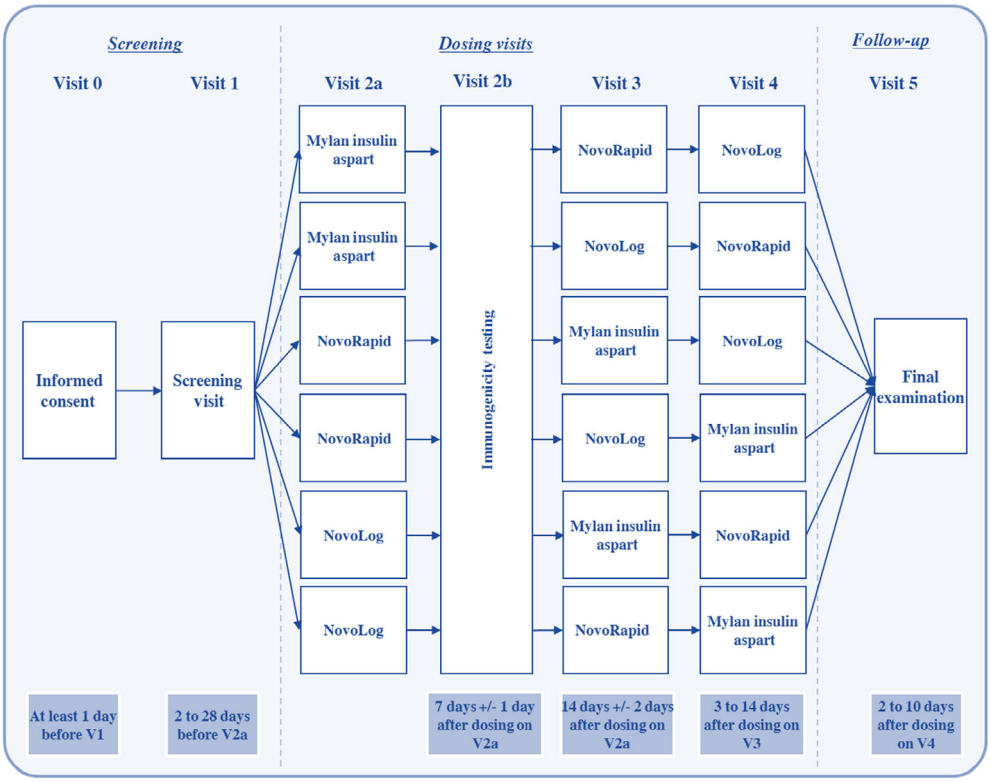

FIGURE 1 Schematic overview of the chronological structure of the study. At first dosing visit (visit 2a [V2a]), subjects were randomized to one of six possible sequences of a single-dose administration of each study drug. The three study drugs were administered in a crossover design with a 12- to 16-day wash-out period after dosing visits 2a and a 3 to 14-day wash-out period after visit 4 (V4). In addition to the current product names NovoLog and NovoRapid, the product code names Ref-InsAsp- US and Ref-InsAsp-EU are used throughout the document, respectively

Secondary PD endpoints were AUC_{GIR} from 0 to 4 hours ($AUC_{GIR,0\text{-}4h}$), from 0 to 6 hours ($AUC_{GIR,0\text{-}6h}$), and from 6 hours until the end of clamp ($AUC_{GIR,6\text{-}last}$); time to maximum glucose infusion rate ($t_{max.GIR}$); time to half-maximum glucose infusion rate before GIR_{max} ($t_{GIR,50\%\text{-}early}$); time to half-maximum glucose infusion rate after GIR_{max} ($t_{GIR,50\%\text{-}late}$, indicator of end of duration of action); time from trial product administration until the BG concentration had decreased by at least 5 mg/dL from baseline (onset of action); and the difference between $t_{GIR,50\%\text{-}late}$ and the onset of action (duration of action).

Safety endpoints included adverse events (AEs), laboratory safety variables (haematology, biochemistry, urinalysis), anti-insulin antibodies (antidrug antibodies [ADAs]), physical examination, vital signs, and ECGs.

2.4 | Assessments

During the clamp procedure, blood was collected predose until 12 hours postdose at prespecified intervals for measurement of glucose, insulin aspart, and C-peptide levels. The blood samples were drawn at predose, and at 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 105, 120, 150, 180, 210, 240, 300, 600, and 720 minutes postdose to determine insulin aspart concentrations. The quantification of insulin aspart in plasma samples employed an immunoaffinity purification method followed by ultraperformance liquid chromatography (UPLC) coupled with tandem mass spectrometric detection (UPLC-MS/MS). The analytical method was validated at inVentiv Health Clinique, Quebec, Canada, over the calibration range of 100 to 8000 pg/mL. Plasma samples fortified with the internal standard (15 N-insulin aspart) were first incubated with a capture reagent consisting of anti-insulin antibodies bound to magnetic beads. The insulin aspart and internal standard present in the solution conjugated with the capture reagent were subsequently immobilized using a strong magnet, while the matrix components were washed away. Insulin aspart and the internal standard were then eluted from the capture reagent, and the resulting extract was analysed via UPLC-MS/MS. The specificity of the mass spectrometric analysis permitted quantification of insulin aspart without interference from endogenous insulin or other insulin analogues.

Glucose levels were measured using the Super GL analyser (manufactured by Dr. Müller Gerätebau, Freital, Germany). The PD response to study drug administration was measured using the euglycaemic clamp method, performed using a glucose clamp device (ClampArt; Profil Neuss, Germany). Predefined target plasma glucose levels were maintained by regulating the GIR automatically. The GIR was recorded every minute using the algorithm implemented into the device. A validated assay was used to measure C-peptide. The quality of clamps was evaluated according to a ClampArt clinical study,[12] with variables determining the precision (defined as the individual coefficient of variation [CV, %] of the BG device measurements during euglycaemia) and the control deviation (defined as the mean absolute difference of individual mean BG measurements from the clamp target level). A variable i.v. infusion of human insulin (15 IU Actrapid [100 IU/mL] in 49 mL saline and 1 mL of the subject's blood) or glucose (diluted with saline before infusion) was initiated to obtain a glucose clamp target level of 81 mg/dL throughout the study.

The presence of ADAs was investigated in predose samples from all subjects at visits 2a (baseline), 2b (6-8 days after initial treatment), and 3 (12-16 days after initial treatment) in a tiered approach that included screening, confirmation, and characterization using a

TABLE 1 Demographic and baseline characteristics per study site (safety analysis set, N = 71)

Variable	Study site 1 (N = 36)	Study site 2 (N = 35)
Gender, male, n (%)	32 (88.9)	30 (85.7)
Gender, female, n (%)	4 (11.1)	5 (14.3)
Race, n (%)		
White	35 (97.2)	35 (100)
Indian	1 (2.8)	0 (0)
Age, y	39.2 (10.41)	35.1 (12.1)
Height, cm	178.4 (7.73)	178.1 (6.4)
Weight, kg	79.14 (9.39)	77.95 (9.2)
Body mass index, kg/m^2	24.81 (1.79)	24.55 (2.3)

Note: All values are expressed as mean (standard deviation) unless otherwise mentioned.

conventional radioimmunoprecipitation assay to detect anti-insulin aspart antibodies in serum samples. Individual samples that were positive in the confirmatory assay were reported as ADA-positive. The relative levels of ADAs in confirmed positive samples were based on the assay response observed in the screening assay. Based on published recommendations,[13,14] subjects were classified as ADA-positive when having either a treatment-induced or treatment-boosted ADA response following administration of the study drug.

2.5 | Sample size and statistical analysis

The sample size was determined based on data from clinical pharmacology studies conducted in healthy subjects available from Ref-InsAsp-US new drug application. The intrasubject coefficients of variation (ISCV) for insulin aspart were estimated to range from 18% to

TABLE 2 Summary statistics and treatment comparisons for pharmacokinetic (PK) endpoints

Primary PK endpoints			
Arithmetic mean (SD)[a]	MYL-1601D (N = 69)	Ref-InsAsp-US (N = 68)	Ref-InsAsp-EU (N = 67)
$AUC_{0\text{-}12h}$ (h*pg/mL)	8188.35 (1489.82)	8017.50 (1286.35)	8015.16 (1180.65)
C_{max} (pg/mL)	3177.57 (939.16)	2984.95 (918.23)	2971.82 (668.99)
Geometric/arithmetic LS-mean ratio[b]	MYL-1601D vs. Ref-InsAsp-US (N = 68)	MYL-1601D vs. Ref-InsAsp-EU (N = 67)	Ref-InsAsp-US vs. Ref-InsAsp-EU (N = 66)
$AUC_{0\text{-}12h}$ (h*pg/mL)	102.17 (100.26; 104.11)	101.84 (100.04; 103.67)	99.67 (97.96; 101.40)
C_{max} (pg/mL)	106.13 (100.71; 111.85)	105.74 (101.09; 110.60)	99.89 (95.46; 104.53)
Secondary PK endpoints: AUC			
Arithmetic mean (SD)	MYL-1601D (N = 69)	Ref-InsAsp-US (N = 68)	Ref-InsAsp-EU (N = 67)
$AUC_{0\text{-}4h}$ (h*pg/mL)	7253.97 (1442.08)	6969.73 (1305.02)	6978.44 (1099.83)
$AUC_{0\text{-}6h}$ (h*pg/mL)	8020.21 (1467.91)	7791.86 (1260.44)	7813.62 (1146.58)
$AUC_{6\text{-}12h}$ (h*pg/mL)	168.14 (224.30)	225.64 (288.91)	201.54 (263.53)
$AUC_{0\text{-}\infty}$ (h*pg/mL)	8288.17 (1490.98)	8137.78 (1276.72)	8123.94 (1193.99)
Geometric/arithmetic LS-mean ratio[b]	MYL-1601D vs. Ref-InsAsp-US (N = 68)	MYL-1601D vs. Ref-InsAsp-EU (N = 67)	Ref-InsAsp-US vs. Ref-InsAsp-EU (N = 66)
$AUC_{0\text{-}4h}$ (h*pg/mL)[a]	104.24 (101.42; 107.11)	104.30 (101.74; 106.86)	100.23 (97.79; 102.68)
$AUC_{0\text{-}6h}$ (h*pg/mL)[c]	103.23 (101.14; 105.34)	102.90 (100.93; 104.84)	99.71 (97.90; 101.52)
$AUC_{6\text{-}12h}$ (h*pg/mL)[c]	75.60 (61.38; 92.16)	81.37 (62.70; 105.75)	107.18 (83.81; 137.45)
$AUC_{0\text{-}\infty}$ (h*pg/mL)[a]	101.87 (100.06; 103.72)	101.74 (99.98; 103.54)	99.71 (98.04; 101.41)
Secondary PK endpoints: time variables			
Arithmetic mean (SD)	MYL-1601D (N = 68)	Ref-InsAsp-EU (N = 67)	Ref-InsAsp-US (N = 66)
t_{max} (h)	1.03 (0.44)	1.10 (0.53)	1.11 (0.45)
$t_{50\%\text{-}early}$ (h)	0.42 (0.10)	0.45 (0.12)	0.46 (0.46)
$t_{50\%\text{-}late}$ (h)	2.98 (0.76)	3.1 (0.84)	3.13 (0.86)
$t_{1/2}$ (h)	0.88 (0.25)	0.89 (0.27)	0.93 (0.33)
λ_z (/h)	0.86 (0.22)	0.86 (0.28)	0.82 (0.27)

Abbreviations: $AUC_{GIR0h\text{-}last}$, area under the glucose infusion rate time curve from 0 hours until the end of clamp; $AUC_{GIR0\text{-}4h}$, AUC from 0 to 4 hours; $AUC_{GIR0\text{-}6h}$, AUC from 0 to 6 hours; $AUC_{GIR6h\text{-}last}$, AUC from 6 to last hours; CI, confidence interval; GIR_{max}, the glucose infusion rate maximum concentration; LS-mean, least square mean; $t_{max,}$ time to maximum observed insulin aspart concentration; $t_{50\%\text{-}early}$, time to half-maximum before $C_{max;}$ $t_{50\%\text{-}late}$, time to half-maximum after C_{max}; $t_{1/2}$, the terminal elimination half-life calculated as $t_{½} = \ln 2/\lambda z$; $\lambda_{z,}$ terminal elimination rate constant of insulin.
[a]Statistical analysis (ANOVA) analysed using log transformation based on the general linear model (proc GLM).
[b]Two-sided 90% confidence intervals.
[c]Fieller's theorem.

26% for various PK and PD primary endpoints.[15,16] Assuming an ISCV of 22% to 26% and an estimated treatment ratio of 0.95 to 1.05, a sample size of 60 completing subjects (10 subjects per sequence) was expected to give the study a power of 95.4%-99% for each comparison (MYL-1601D vs. Ref-InsAsp-EU, and MYL-1601D vs. Ref-InsAsp-US), or a combined study power of at least 91%.

Statistical analyses on the primary PK and PD endpoints were performed using logarithm-transformed data using analysis of variance (ANOVA) based on a general linear model (proc GLM). If the 90% confidence intervals (CIs) for primary PK/PD endpoints lay within 80% to 125% then the PK bioequivalence would be shown for the relevant reference product. If the 90% CIs of the MYL-1601D versus Ref-InsAsp-US comparison lay within 80% to 125%, then the PD similarity would be shown as per the US Food and Drug Administration (FDA) recommendation. If the 95% CIs of the MYL-1601D versus Ref-InsAsp-EU comparison lay within 80% to 125%, then the PD similarity would be shown as per the EMA recommendation. The data were analysed using SAS version 9.3 for PK and SAS version 9.4 for PD, and safety variables. The treatment ratios and 90% or 95% CI for secondary PK and PD endpoints were conducted using Fieller's theorem. The time endpoints t_{max}, $t_{50\%\text{-early}}$, $t_{50\%\text{-late}}$, $t_{½}$, and λ_z were analysed using summary statistics by treatment only.

Based on C-peptide concentrations, the following experiments were excluded from the PD assessments in sensitivity analyses: (a) clamps with a baseline C-peptide concentration up to 0.5 nmol/L (median of the values at $t = -30$, -15, and -2 minutes) if postdosing C-peptide concentrations increased to 1 nmol/L or higher; and (b) clamps with a baseline C-peptide concentration of more than 0.5 nmol/L if postdosing C-peptide concentrations increased to at least 100% of baseline values. If increases in C-peptide concentrations above the cut-off of 1 nmol/L or 100% of baseline value were based on the rules set up above, then the PD variables were excluded in the sensitivity analysis only. A total of three sensitivity analyses were performed. The first one excluded all subject profiles that met the criteria, the second one excluded all subject profiles that met the criteria in the last 6 hours, and the third sensitivity analysis excluded all subject profiles that met the criteria in the first 6 hours. In addition, a sensitivity analysis was conducted to calculate the 90% or 95% CI for the ratio of arithmetic LS-means of the primary PD endpoints. Safety results were presented using descriptive statistics by visit/treatment.

The clamp quality variables were calculated based on all measurements during the 12-hour clamp procedure, where the start point was the first time that the GIR was more than 0 after dosing and the endpoint was the last time the GIR was more than 0.

3 | RESULTS

3.1 | Study population

Seventy-two healthy subjects (36 at the Neuss study site and 36 at the Mainz study site) were randomized, of whom 71 were exposed to the study drugs (70 to MYL-1601D, 69 to Ref-InsAsp-US, and 67 to Ref-InsAsp-EU) and 66 completed the study. Six subjects discontinued before randomization, as scheduling two subjects for a clamp procedure was not possible within the required time window (the dosing day could only be rescheduled once), which led to discontinuation, and four subjects withdrew their consent, one of them prior to the first dosing. Overall, the PK analysis comprised 68 subjects for MYL-1601D compared with Ref-InsAsp-US, 67 subjects for MYL-1601D compared with Ref-InsAsp-EU, and 66 subjects for Ref-InsAsp-US compared with Ref-InsAsp-EU. The PD analysis comprised 67 subjects each for MYL-1601D compared with Ref-InsAsp-US and Ref-InsAsp-EU, and 65 subjects for Ref-InsAsp-US compared with Ref-InsAsp-EU.

A summary of the demographics and clinical baseline characteristics is presented for the 71 subjects at the two study sites in Table 1.

3.2 | PK results

The mean plasma concentration-time profiles of insulin aspart of MYL-1601D, Ref-InsAsp-US, and Ref-InsAsp-EU were similar throughout the treatment (Figure S1). The primary PK variables were comparable for all three study drugs. For the primary endpoints, the PK bioequivalence criterion was met for both MYL-1601D to Ref-InsAsp-US, and for MYL-1601D to Ref-InsAsp-EU, with the 90% CIs of the geometric LS-mean ratio of treatments being within 80% to 125%.

The secondary PK endpoints were comparable, with minor differences between MYL-1601D and Ref-InsAsp-EU, and between MYL-1601D and Ref-InsAsp-US. Mean values were higher for MYL-1601D when compared with Ref-InsAsp-EU and Ref-InsAsp-US during early absorption ($AUC_{0\text{-}4h}$ and $AUC_{0\text{-}6h}$). In the second half of the clamp ($AUC_{6\text{-}12h}$), the mean values of MYL-1601D were lower than those of Ref-InsAsp-EU and Ref-InsAsp-US. The $AUC_{0\text{-}\infty}$ was comparable for all treatments (mean ranging from 8124 to 8288 h*pg/mL) and similar to the primary PK endpoint $AUC_{0\text{-}12h}$ (Table 2). $AUC_{0\text{-}\infty}$, $AUC_{0\text{-}4h}$, and $AUC_{0\text{-}6h}$ showed close similarity, as indicated by LS-mean ratios showing differences of less than 5% and 90% CIs falling within 80% to 125% for MYL-1601D compared with Ref-InsAsp-US and Ref-InsAsp-

TABLE 3 Summary statistics of clamp quality data

	MYL-1601D (N = 69)	Ref-InsAsp-EU (N = 67)	Ref-InsAsp-US (N = 68)
Precision (CV, %)			
Mean (SD)	5.47 (1.983)	5.57 (1.652)	5.55 (2.049)
Min, max	2.8, 12.3	2.5, 10.5	2.1, 15.9
Deviation from target (mg/dL)			
Mean (SD)	0.51 (0.584)	0.42 (0.477)	0.51 (0.594)
Min, max	−0.6, 3.1	−0.5, 1.9	−0.4, 3.0

Abbreviations: CV, coefficient of variation; SD, standard deviation.

TABLE 4 Summary statistics and treatment comparisons for pharmacodynamic (PD) endpoints

Primary PD endpoints			
Arithmetic mean (SD)	MYL-1601D (N = 69)	Ref-InsAsp-US (N = 67)	Ref-InsAsp-EU (N = 67)
$AUC_{GIR,0\text{-}last}$ (mg/kg)	2773.4 (811.2)	2741.3 (812.5)	2869.3 (889.5)
GIR_{max} (mg/kg/min)	9.846 (3.1909)	9.716 (3.0834)	10.238 (3.0175)
Geometric/arithmetic LS-mean[a]	MYL-1601D vs. Ref-InsAsp-US (N = 67)	MYL-1601D vs. Ref-InsAsp-EU (N = 67)	Ref-InsAsp-US vs. Ref-InsAsp-EU (N = 65)
$AUC_{GIR,0h\text{-}last}$ (mg/kg)	99.93 (95.74; 104.30)[b]	96.42 (91.17; 101.98)[c]	96.10 (92.37; 99.99)[b]
GIR_{max} (mg/kg/min)	100.12 (94.46; 106.12)[b]	95.10 (89.37; 101.19)[c]	94.41 (89.38; 99.74)[b]
Secondary PD endpoints			
Arithmetic mean (SD)	MYL-1601D (N = 69)	Ref-InsAsp-US (N = 67)	Ref-InsAsp-EU (N = 67)
$AUC_{GIR,0\text{-}4h}$ (mg/kg)	1595.3 (512.2)	1516.7 (507.4)	1629.3 (534.9)
$AUC_{GIR,0\text{-}6h}$ (mg/kg)	2190.9 (673.4)	2125.1 (667.2)	2260.5 (730.4)
$AUC_{GIR,6h\text{-}last}$ (mg/kg)	582.5 (256.7)	616.3 (274.7)	608.8 (284.0)
Geometric/arithmetic LS-mean ratio[d]	MYL-1601D vs. Ref-InsAsp-US (N = 67)	MYL-1601D vs. Ref-InsAsp-EU (N = 67)	Ref-InsAsp-US vs. Ref-InsAsp-EU (N = 65)
$AUC_{GIR,0\text{-}4h}$ (mg/kg)	103.31 (99.27; 107.53)[b]	97. 69 (93.60; 101.95)[c]	94.11 (90.37; 97.99)[b]
$AUC_{GIR,0\text{-}6h}$ (mg/kg)	101.37 (97.88; 104.98)[b]	96.37 (92.42; 100.49)[c]	94.81 (91.46; 98.27)[b]
$AUC_{GIR,6h\text{-}last}$ (mg/kg)	93.33 (88.79; 98.09)[b]	94.77 (87.27; 102.87)[c]	101.05 (95.75; 106.64)[b]

Abbreviations: $AUC_{GIR0h\text{-}last}$, area under the glucose infusion rate time curve from 0 hours until the end of clamp; $AUC_{GIR0\text{-}4h}$, AUC from 0 to 4 hours; $AUC_{GIR0\text{-}6h}$, AUC from 0 to 6 hours; $AUC_{GIR6h\text{-}last}$, AUC from 6 to last hours; CI, confidence interval; GIR_{max}, the glucose infusion rate maximum concentration.

[a]Statistical analysis (ANOVA) analysed using log transformation based on the general linear model (proc GLM).

[b]Two-sided 90% confidence intervals.

[c]Two-sided 95% confidence intervals.

[d]Fieller's theorem.

EU. For the variable $AUC_{6\text{-}12h}$, the lower bound of the 90% CI exceeded the lower limit of 80%. Early, late, and maximum exposure to insulin were comparable, as were the terminal elimination half-life and the terminal elimination rate constant of insulin.

3.2.1 | Quality of clamps

The precision and deviation results show that the clamp quality was good and comparable among the three study drugs. Mean precision was less than 6.00% and mean deviation from the target was close to 0.5 mg/dL. One Ref-InsAsp-US subject profile did not meet the quality clamp criteria (the clamp CV was <15% and the mean clamp deviation from target was within range of ±10 mg/dL) and was excluded from the PD comparison. Table 3 summarizes the two clamp quality variables for the different treatments and the clamp periods with active GIR.

3.3 | PD results

The mean GIR profiles after administration of MYL-1601D, Ref-InsAsp-US, and Ref-InsAsp-EU were similar throughout the treatment (Figure S2). For the primary PD variables, $AUC_{GIR,0\text{-}last}$, and GIR_{max}, the 90% CIs of the geometric LS-mean ratio of the comparison of MYL-1601D versus Ref-InsAsp-US, and Ref-InsAsp-US versus Ref-InsAsp-EU, and the 95% CIs of the comparison of MYL-1601D versus Ref-InsAsp-EU, were within 80% to 125%, and PD bioequivalence among all three insulins was displayed (Table 4).

For the secondary PD endpoints $AUC_{GIR,0\text{-}4h}$, $AUC_{GIR,0\text{-}6h}$, and $AUC_{GIR,6\text{-}last}$, the 90% CIs of the comparison of MYL-1601D versus Ref-InsAsp-US, and of Ref-InsAsp-US versus Ref-InsAsp-EU, as well as 95% CIs of the comparison of MYL-1601D versus Ref-InsAsp-EU, were within 80% to 125%, showing close similarity. From the summary statistics, the secondary PD time endpoints $t_{max.GIR}$, $t_{GIR,50\%\text{-}early}$, and $t_{GIR,50\%\text{-}late}$, as well as onset and duration of action, were comparable for MYL-1601D, Ref-InsAsp-EU, and Ref-InsAsp-US, as indicated by comparable means (Table 4).

A sensitivity analysis based on arithmetic means confirmed equivalence for the primary PD endpoints. All three C-peptide sensitivity analyses showed that the 90% CIs of $AUC_{GIR,0\text{-}last}$ and GIR_{max} were within 80% to 125% and underlined that exclusion because of C-peptide criteria did not impact the bioequivalence results. The result of the C-peptide concentration against time is presented in Figure S3.

3.4 | Safety

Overall, 11 subjects (15.7%) after MYL-1601D (18 events), 13 subjects (19.4%) after Ref-InsAsp-EU (13 events), and 11 subjects (15.9%) after

Ref-InsAsp-US (12 events) administration experienced at least one treatment-emergent adverse event (TEAE). All TEAES were of mild or moderate intensity (except for one severe TEAE that was not related to the study drug). There were no serious AEs or death, and none of the AEs resulted in discontinuation of the study drug. The most frequent TEAEs were headache (four [5.7%] subjects after MYL-1601D, eight [11.9%] subjects after Ref-InsAsp-EU, and five [7.2%] subjects after Ref-InsAsp-US administration), nasopharyngitis (three [4.3%] subjects after MYL-1601D, two [3.0%] subjects after Ref-InsAsp-EU, and none after Ref-InsAsp-US administration), injection site erythema (two [2.9%] subjects after MYL-1601D, one [1.5%] subject after Ref-InsAsp-EU, and one [1.4%] subject after Ref-InsAsp-US administration). Other less frequent TEAEs were hypoglycaemia with two events in two subjects, each one after MYL-1601D and Ref-InsAsp-US administration.

Immunogenicity assessments did not show an increased ADA reactivity for MYL-1601D when compared with Ref-InsAsp-US or Ref-InsAsp-EU, because the majority of subjects lacked any detectable immunogenicity response against insulin aspart or had relevant antibody levels predose with no treatment boosting. Of 72 subjects, five were confirmed to be positive-ADA at any time point during the study, while four (5.6%) subjects were already positive at baseline, prior to dosing. Overall, the results of the immunogenicity profiles were comparable between the treatment groups. There were no clinically significant abnormal findings or changes in laboratory test results, vital signs, ECG, and physical examination.

4 | DISCUSSION

This was a single-dose euglycaemic glucose clamp study in healthy subjects designed to evaluate the PK and PD variables of MYL-1601D, Ref-InsAsp-US, and Ref-InsAsp-EU after subcutaneous administration of insulin at a dose of 0.2 U/kg body weight. Based on the findings of primary PK endpoints ($AUC_{0\text{-}12h}$ and C_{max}), the PK bioequivalence of MYL-1601D versus US Ref-InsAsp-US and EU Ref-InsAsp-EU was shown in this study. Comparable PK/PD results between Ref-InsAsp-US and Ref-InsAsp-EU were also shown.

Although the secondary PK endpoints were not required to fulfill the standard bioequivalence criteria. The 90% CIs of the LS-mean ratios of the secondary PK endpoints $AUC_{0\text{-}4h}$, $AUC_{0\text{-}6h}$, and $AUC_{0\text{-}\infty}$ were within 80% to 125% for all three comparisons. But the 90% CIs for $AUC_{6\text{-}12h}$ exceeded the upper limit of bioequivalence criteria. This variable showed high variability, primarily as a result of very low responses over this trailing time interval. About half of the subjects had no concentration after 6 hours, which further reduced the power for this variable. The corresponding PD variable ($AUC_{GIR,6h\text{-}last}$) for this interval also met the predefined equivalence criteria. The PD characteristics of MYL-1601D, Ref-InsAsp-EU, and Ref-InsAsp-US further supported the conclusions drawn from the PK characteristics, with similarly fast onset and offset of glucose-lowering effects and a comparable onset and duration of action. Overall, the results of the primary PD endpoints were robust, as shown in sensitivity analyses using Fieller's theorem or excluding profiles exceeding predefined C-peptide limits.

All the three study drugs were well tolerated, with headache and nasopharyngitis being the most frequent TEAEs. The risk of hypoglycaemia was minimized in this study, as insulin administration occurred in a medically supervised environment and a clamp device kept the subject's BG level constant at 81 mg/dL with only slight deviations for 12 hours after dosing. Further, immunogenicity assessment did not raise any safety concerns because there was only one transient treatment-induced ADA reaction.

This clinical study used the classical three-period crossover design to show the similar nature of biological products in terms of safety and efficacy. Each subject acted as their own control, and also compared the two reference products with each other based on the guidelines on the clinical development of human insulin and insulin analogues.[17,18] The study design was consistent with EMA 2015 guidelines on recombinant human insulin and insulin analogues.[17] Relevant general considerations from the FDA guidance for industry (December 2016) on biosimilarity to a reference product[17] were followed in this study. The dose of 0.2 U/kg body weight was used as per the guideline recommendations for testing fast-acting insulin preparations, enabling provision of a robust dose-response relationship in healthy subjects.[19] The euglycaemic glucose clamp was chosen, and it is a validated method to ensure a constant BG predetermined level for the PD effect of a glucose-lowering drug, such as exogenous insulin.[19,20] A clamp duration of 12 hours was selected to assess the complete PD and PK profiles of a single dose and to assess the duration of action. To achieve the highest clamp quality, the clamp setting was based on an automated glucose clamp technique with continuous BG measurements and where minute-by-minute adaptations of glucose infusion rates were possible. Additionally, the euglycaemic clamp technique helped to minimize the risk of any drug-induced hypoglycaemia, and is recommended by the EMA for showing the biosimilarity between insulins in clinical studies. The study findings showed that mean precision was less than 6.00% and the mean deviation from target was close to 0.5 mg/dL, indicating low BG variation and a high-quality euglycaemic clamp technique throughout. Thus, in accordance with quality variables (precision and deviation from target), as reported in the literature,[8] our results showed that the clamp quality was good and comparable among the three treatments.

The study had a predominantly male population and, except for one Asian subject, all the subjects were White/Caucasian and from Europe, which could be a possible limitation. As per the guideline's recommendations, the inclusion of only men in the studies is preferable, as insulin sensitivity in women may vary during the menstrual cycle.[17,18]

In conclusion, this study showed bioequivalence between the MYL-1601D biosimilar, Ref-InsAsp-US, and Ref-InsAsp-EU, as measured by the primary PK and PD endpoints. Overall, all three study

drugs were well tolerated, with no significant safety issues. Further, the study established a scientific bridge between Ref-InsAsp-US and Ref-InsAsp-EU, allowing a phase 3 study in T1D, only with the Ref-InsAsp-US (NovoLog) as a comparator.

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CONFLICT OF INTEREST

UH is an employee of Profil. YR, ML, CD, TL, GR, and AB are employees of Viatris Inc. and may hold stock or stock options in the company. GCL, AC, and NS are Biocon Biologics Ltd employees and may hold stock or stock options in the company.

AUTHOR CONTRIBUTIONS

UH, YR, AC, ML, TL, GR, and AB contributed to the design of the study, the acquisition, analysis, and interpretation of data, and critically revised the manuscript for important intellectual content. CD, NS, and GCL contributed to the acquisition and analysis of data. All the authors reviewed and approved the manuscript for publication.

DATA SHARING STATEMENT

The datasets that support the findings of this study are available from the corresponding author, YR, upon reasonable request.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The datasets that support the findings of this study are available from the corresponding author, Dr. Yaron Raiter, upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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